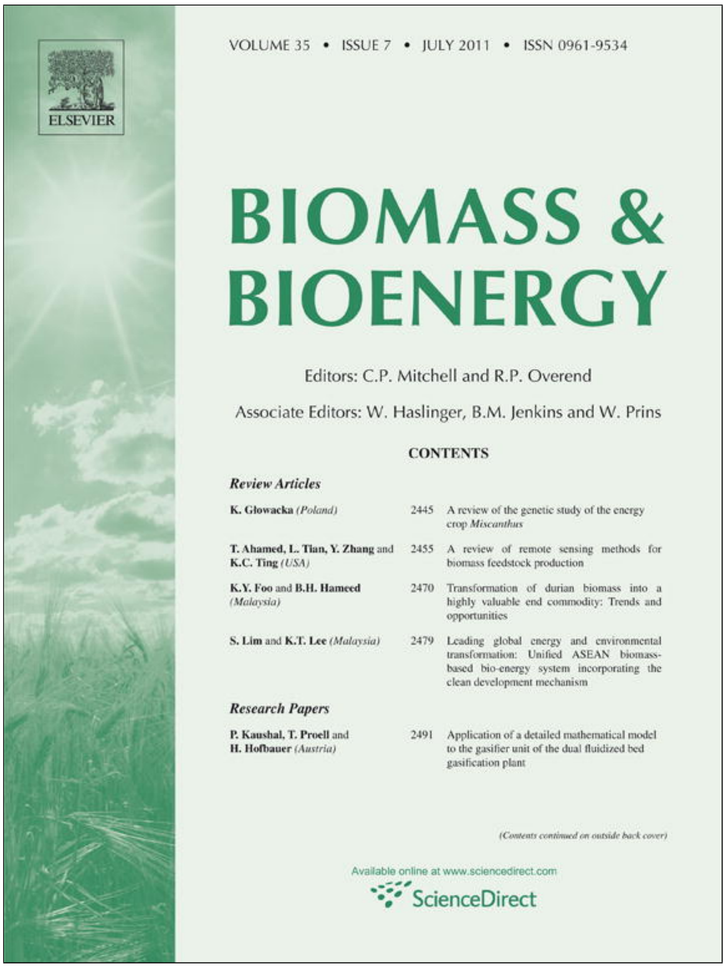

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Phytochemicals of selected plant species of the Apocynaceae and Asclepiadaceae from Western Ghats, Tamil Nadu, India

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ABSTRACT

A concern about the declining supply of petroleum products has led to a renewed interest in evaluating plant species as potential alternate sources of energy. Five species of the Apocynaceae and three species of the Asclepiadaceae from the Western Ghats were evaluated as alternative sources of energy, oil, polyphenol, and phytochemicals. The highest protein content was observed in *Caralluma attenuata* with 6.6%. Plant samples of *Aganosma cymosa* yielded 10.3% oil. The highest polyphenol content was observed in *Carissa carandas* with 7.7%. Species of the Asclepiadaceae exhibited the highest quantity of hydrocarbon viz. *Sarcostemma brevistigma* (3.6%), *C. attenuata* (3.4%), and *Tylophora asthmatica* (2.7%). Carbohydrate content was high in *S. brevistigma* with 6.9%. A whole plant gross calorific value of 16.5 MJ kg^{-1} for *Nerium odorum* (white flower variety) was the highest value observed. The highest gross calorific value observed in the oil fraction was from *C. attenuata* with 33.4 MJ kg^{-1}. The hydrocarbon fraction of *T. asthmatica* exhibited the highest gross calorific value of 39.2 M Jkg^{-1}. The hydrocarbon fractions were further analyzed using NMR to determine the type of isoprene present. Fatty acid compositions of oil samples were also analyzed. All the species except *N. odorum* (white flower variety) contained higher quantities of saturated fatty acids than unsaturated fatty acids.

1. Introduction

People need fossil fuels for their various activities. Today, with the decline in readily available petroleum products and without the discovery of additional reserves or resources, it is estimated that natural gas will be exhausted by the year 2047, oil by the year 2080, and coal and lignite by the year approximately 2180. Spiraling costs of liquid fuels and chemical feedstocks have renewed an interest and awareness of the potential value of underutilized and diverse plant species as carbon sources. During the mid to late 1970's, scientists rediscovered that plant biomass was a renewable resource capable of providing non-polluting safe fuels and began researching biomass again [1,2]. Plants with an accumulation of useful products like latex, gums, and resins are proven to be excellent resource materials.

It was suggested by Melvin Calvin that the species belonging to the Asclepiadaceae and Euphorbiaceae families deserve special attention because they contain oil and hydrocarbon rich in latexes [3]. Many of these species are capable of growing with minimal water requirement with no agronomic management in unused wastelands and can be established through stem cuttings. Species that yield large quantities of either one or two phytochemicals are recommended for large-scale cultivation, enabling employment opportunity for tribal communities. "Energy farms" or

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"Petroleum plantations" contain plants that produce hydrocarbons that can substitute for petroleum fuels and some of the costly petroleum feedstocks. The remaining biomass can then be used to manufacture paper, to produce power by direct combustion, as animal feed, or to manufacture construction materials including insulations and soil amendments. The compilation of phytochemical data for Indian plants will serve as a ready reference for identifying promising plant species for future consideration for large-scale cultivation of non- conventional agricultural plants for biomass plantations in unused marginal lands and waste areas.

Whole plant oils are distributed throughout the plant and are a valuable mixtures of a wide variety of chemical intermediates like sterols, long chain alcohols, resins, fatty acids, esters, waxes, terpenes, and other hydrocarbons [4–7]. They may also be used in wax and polish formulations [8]. Plant oils are used for food products, while large quantities are used in industrial applications [9]. Vegetable oils have great potential as reliable and renewable sources of fuel for compression engines.

Natural rubber is the most common hydrocarbon polymer found in green plants. Low molecular weight natural rubber would be of interest as a plasticizing additive (processing aid) to rubber mixes, for making cements (adhesives), and if low enough in cost, as a hydrocarbon feedstocks. Trans-polyisoprene (gutta) can have large-scale applications as both additional thermoplastic and thermosetting resin if available at prices competitive with natural rubber and synthetic polymer [6].

Hydrocarbons in plants, such as natural rubber (polyisoprene), have chemical structures similar to many hydrocarbons derived from petroleum (MW 10,000), but with molecular weights in the order of 5,000,000 to 20,000,000. Such materials when cracked will produce hydrocarbons of lower molecular weight which can be used as alternative energy sources for fuel and/or chemical raw materials that are used in manufacturing of a large number of additional products [10].

The objective of the present study was to quantify some of the phytochemicals of selected species of the Asclepiadaceae and Apocynaceae families from Western Ghats, Tamil Nadu, India.

2. Materials and methods

2.1. Plant materials

Five species belonging to Apocynaceae and three species belonging to Asclepiadaceae were collected from Courtallum and Srivilliputhur of Western Ghats, Tamil Nadu State, India (Table 1). Courtallum (8.92170 °N, 77.2786 °E) is a city located in the Tirunelveli District at an elevation of 160 km. Srivilliputhur (9.5161 °N, 77.6300 °E) is located 85 km northeast of Courtallum at an elevation of 146 m. Both cities are located in the Virudhungar District in the Indian State of Tamil Nadu. The climate of the region is semiarid tropical monsoon type. All the species analyzed, except *C. attenuata* which is a herb, are climber shrubs or small trees (*A. cathartica* – climbing shrub; *Allamanda cymosa* – climber; *C. carandas* – thorny shrub or small tree; *N. odorum* – large shrub; *S. brevistigma* – shrub; and *T. asthmatica* – climber) with potential fiber utility value and suitable for annual pollarding. *S. brevistigma*, and *T. asthmatica* are restricted to foot hill regions of Western Ghats. All other species grow in dry waste land areas and are distributed throughout Tamil Nadu.

Healthy plants of approximately the same age, similar height, and diameter were randomly collected from the same agro-climatic zones during the spring season. When collecting whole plant samples, herbaceous, small woody plants, and climbers were clipped at ground level including the leaves and flowers. Samples were allowed to dry in a protected sheltered area at ambient summer temperatures. After thoroughly drying, the samples were ground in a Wiley mill equipped with a 1 mm diameter sieve. A minimum of 15 populations of each species were collected with each consisting of 10–20 plants composited into one sample with a total fresh weight of 2000–2500 g for chemical analysis. Each sample was sub-sampled twice.

2.2. Extraction of chemical constituents

Extractable fractions were removed from each sample with propan-2-one followed by n-hexane in a Soxhlet apparatus for a minimum of 24 h per solvent. The propan-2-one extract was allowed to dry at 45 °C for 48 h and partitioned between

Table 1 – Composition of chemical constituents of whole plant samples of species from the Western Ghats, India.

Species	Extractable yields[a]			
	Protein (%)	Oil (%)	Polyphenol (%)	Hydrocarbon (%)
Apocynaceae				
Allamanda cathartica	1.4 ± 0.80	1.3 ± 0.27	4.0 ± 0.29	1.3 ± 0.17
Aganosma cymosa	1.5 ± 0.20	10.3 ± 0.26	1.7 ± 0.16	1.2 ± 0.12
Carissa carandas	2.7 ± 0.13	5.8 ± 0.16	7.7 ± 0.27	1.7 ± 0.18
Nerium odorum (red flower variety)	2.7 ± 0.28	5.0 ± 0.26	6.4 ± 0.24	1.1 ± 0.15
Nerium odorum (white flower variety)	4.1 ± 0.19	4.0 ± 0.25	5.0 ± 0.29	1.0 ± 0.25
Asclepiadaceae				
Caralluma attenuata	6.6 ± 0.24	2.7 ± 0.26	3.8 ± 0.26	3.4 ± 0.28
Sarcostemma brevistigma	3.4 ± 0.25	2.1 ± 0.12	4.2 ± 0.21	3.6 ± 0.27
Tylophora asthmatica	2.1 ± 0.23	1.8 ± 0.16	3.1 ± 0.15	2.7 ± 0.29

a All values are the mean of three replicates with the standard deviation.

Table 2 – Ash, lignin, and carbohydrate contents of whole plant samples of species from the Western Ghats, India.

Species	Ash[a] (%)	Lignin[a] (%)	Carbohydrate[a] (%)	Total[b] (%)
Apocynaceae				
Allamanda cathartica	0.7 ± 0.11	57.7 ± 0.22	2.3 ± 0.12	68.7
Aganosma cymosa	0.5 ± 0.06	20.0 ± 0.15	1.7 ± 0.21	36.9
Carissa carandas	2.7 ± 0.10	47.0 ± 0.20	1.5 ± 0.13	69.1
Nerium odorum (red flower variety)	0.5 ± 0.04	51.1 ± 0.20	3.0 ± 0.49	69.8
Nerium odorum (white flower variety)	0.7 ± 0.03	30.8 ± 0.24	1.1 ± 0.29	46.7
Asclepiadaceae				
Caralluma attenuata	1.1 ± 0.13	22.8 ± 0.21	3.0 ± 0.31	43.4
Sarcostemma brevistigma	0.04 ± 0.03	57.7 ± 0.11	6.9 ± 0.19	77.94
Tylophora asthmatica	1.1 ± 0.03	42.8 ± 0.22	4.2 ± 0.25	57.8

a All values are the mean of three replicates with the standard deviation.
b Total = protein + oil + polyphenol + hydrocarbon + ash + lignin + carbohydrate.

n-hexane and aqueous ethanol (water: ethanol, 1:7) to obtain fractions of "oil" and "polyphenol" which were oven dried at 45 °C for 48 h and weighed for yield. The 'hydrocarbon' fraction was also oven dried at 40 °C for 36 h and weighed for yield after removal of the n-hexane [4,5].

2.3. *Analytical analysis*

Ground sub-samples were analyzed for ash and lignin content [11]. Protein content was determined by Kjeldahl method [12]. Carbohydrate content was quantified according to Dubois et al. [13].

NMR spectra of hydrocarbon fractions were recorded using a Bruker AC 300F NMR spectrometer (300 MHZ) with tetramethylsilane (TMS) as the internal standard and (deuterochloroform) $CDCl_3$ as the solvent. The most useful resonances for analytical purposes are the methyl peaks at 1.59 ppm for 3,4 units and 1.05 ppm for 1,2 units [14,15].

Gross calorific values of plant samples, oil fractions, and hydrocarbon fractions were determined using a Toshniwal, Model cc.0.1, Bomb calorimeter [16].

Fatty acid composition of the oil fractions were analyzed using methyl esters of the fatty acids and a gas liquid chromatograph equipped with an SE-30 column [17].

2.4. *Statistical analysis*

Three replications of each species sample were evaluated for extraction of chemical constituents, protein, ash content, and gross calorific values. Values in Tables 1, 2 and 3 are the means of three replications with a standard deviation (SD).

3. Results and discussion

3.1. *Chemical composition of constituents*

Protein, oil, polyphenol, and hydrocarbon yields are shown in Table 1. *C. attenuata* had 6.6% protein followed by *N. odorum* (red flower variety) with 4.1%. Three species had more than 3% protein content. If the species rich in protein are properly handled and processed in order to remove all the anti-nutritional factors especially glucosides, phytates [18,19], alkaloids, and saponins [20] that complicate their use as animal feed, then they would have the potential to serve as a protein rich food for cattle [21].

Whole plant samples of *A. cymosa* yielded 10.3% oil followed by *C. carandas* with 5.8%, and *N. odorum* (red flower variety) with 5.0%. An oil concentration of 6.8% was previously reported for

Table 3 – Gross calorific values of oil and hydrocarbon of whole plant samples of species from the Western Ghats, India.

Species	Gross calorific value[a]		
	Whole plant sample [MJ kg^{-1} (dry)]	Oil [MJ kg^{-1} (dry)]	Hydrocarbon [MJ kg^{-1} (dry)]
Apocynaceae			
Allamanda cathartica	16.2 ± 0.06	30.8 ± 0.07	33.0 ± 0.05
Aganosma cymosa	14.9 ± 0.18	29.3 ± 0.08	35.8 ± 0.20
Carissa carandas	15.8 ± 0.13	31.5 ± 0.06	34.4 ± 0.20
Nerium odorum (red flower. variety)	15.9 ± 0.07	33.2 ± 0.05	37.6 ± 0.05
Nerium odorum (white flower variety)	16.5 ± 0.05	32.7 ± 0.06	37.2 ± 0.07
Asclepiadaceae			
Caralluma attenuata	12.5 ± 0.17	33.4 ± 0.18	38.9 ± 0.16
Sarcostemma brevistigma	13.3 ± 0.09	33.3 ± 0.04	36.5 ± 0.04
Tylophora asthmatica	15.2 ± 0.08	31.7 ± 0.12	39.2 ± 0.14

a All values are the mean of three replicates with the standard deviation.

C. carandas [22]. The appearance of the plant oil fractions was dark with a melting property at slightly above room temperature (20 °C) turning into a low viscosity fluid. Plant oils are potential sources of industrial feedstocks and alternatives for conventional oils. *A. cymosa* with 10.3% oil could potentially be used in the future as an alternate to conventional oil if properly handled to reduce their viscosity [9].

Whole plant samples contained 1.7%–7.7% polyphenols. *C. carandas* had the highest quantity of polyphenols with 7.7%, while *A. cymosa* had the lowest with 1.7%. A polyphenol concentration of 10.4% for *C. carandas* was previously reported [22]. Polyphenol fractions can be a mixture of phytochemicals including a variety of lipids, tannins and phlobaphenes [23]. In the future, these types of constituents may contribute substantially to the manufacturing of various adhesives, phenolic resins, and antioxidants [4–6]. The polyphenol fraction extracted with propan-2-one contained 50–60% carbon and had a higher calorific value than methanol. However, the latter was considerably lower than that in whole plant oils, hydrocarbons, or petroleum [8].

The hydrocarbon content ranged from 1.0% to 3.6%. *S. brevistigma* had the highest quantity with 3.6% followed by *C. attenuata* with 3.4% and *T. asthmatica* with 2.7%. All of the species sampled yielded 1% or more. Trans-polyisoprene components of hydrocarbons though low in concentration and molecular weight may be used for rubber adhesive products or as a hydrocarbon feedstocks [6].

All the plant samples had ash contents below 1% except for *C. carandas* (2.7%), *C. attentuata* (1.1%), and *T. asthmatica* (1.1%) (Table 2). The ash content directly affects the quality of the fuel. Low ash content is a positive attribute for a potential fuel since high ash content has a negative effect on the calorific value [24].

Lignin content ranged from 20.0% in *A. cymosa* to 57.7% in *A. cathartica* (Table 2). A high lignin value increases the heat value of a species, but at the same time it decreases its digestibility as forage, and increases its vulnerability to insects, fungi, and bacterial threats [25].

The whole plant samples contained 1.1%–6.9% carbohydrate (Table 2). *S. brevistigma* had the highest quantity of carbohydrate with 6.9% while the lowest was *N. odorum* with only 1.1%. The difference between the reported compounds and the total mass balance could be accounted for by starch, cellulose, hemicellulose, minerals, and unknown compounds.

3.2. *Gross calorific value*

The quality of a fuel is determined by the amount of heat generated from a unit mass of fuel (MJ kg^{-1}). The calorific value is considered an important parameter for comparing one fuel to another. The gross calorific values of the whole plant samples, oil, and hydrocarbon fractions indicate that they might be potentially useful as an intermediate energy sources (Table 3). The gross calorific value of plant samples ranged from 12.5 to 16.5 MJ kg^{-1}. Two species had calorific value above 16.2 M Jkg^{-1}. The calorific value of *N. odorum* (white flower variety) was 16.5 MJ kg^{-1}, which is higher than that of rice straw hulls and lignite coal (16.2 MJ kg^{-1}) and comparable to cattle manure (17.2 MJ kg^{-1}). Sekar and Francis [22] reported a gross calorific value of 18.5 MJ kg^{-1} for *C. carandas* compared to a value of 15.8 MJ kg^{-1} in the present study.

The higher ash content and lower lignin content is reflected in the low caloric value of *C. attenuata* confirming the conclusions of Van Emon and Seiber [26], since it has a comparatively low calorific value compared to the other species. Even though *C. carandas* had high ash content, it exhibited a calorific value of 15.8 MJ kg^{-1} which could be attributed to the amount of lignin present in the sample. The gross calorific value is also dependent on the amount of proteins, oils, polyphenols, and hydrocarbons present in the plant tissue.

The gross calorific values of the oil fractions varied from 29.3 to 33.4 MJ kg^{-1}. All the species, expect *A. cymosa* had a gross calorific value greater than anthracite coal with 29.7 MJ kg^{-1}. The low gross calorific value of the oil fractions compared to crude oil might be due to the presence of unsaturated fatty acids. This agrees with the conclusions of Goering et al. [27] that fuel properties of vegetable oils with fewer hydrogen atoms, have a greater unsaturation which decreases the gross calorific value.

The gross calorific value of hydrocarbon fraction ranged from 33.0 to 39.2 MJ kg^{-1}. The calorific value of all the species is higher than the calorific value of anthracite coal. The gross calorific value of *T. asthmatica* with 39.2 MJ kg^{-1}, and *C. attenuata* with 38.9 MJ kg^{-1}, both higher than of anthracite coal and also comparable with that of Mexican fuel oil with 43.1 MJ kg^{-1}. The gross calorific value of the hydrocarbon fraction is dependent on the composition of the substances such as rubber, gutta, and their molecular weights.

3.3. *NMR spectroscopy*

Cis–methyl was observed at 1.63 ppm and trans-methyl at 1.53 ppm. The hydrocarbon fraction of *A. cathartica* and *C. carandas* had cis-methyl with the 1,2 moiety. *A. cymosa*, *N. odorum* (red flower variety), *C. attenuata* and *T. asthmatica* had cis-methyl with 1,2 + 3,4 moieties. *N. odorum* (white flower variety) had only cis-methyl. The only species having trans-methyl with 1,2 + 3,4 moieties was *S. brevistigma*. Isoprene biosynthesis is one of the principle pathways in the formation of latex. Polyisoprene is the main constituents of latex which varies in quantity and weight from species to species.

3.4. *Fatty acid composition*

The fatty acid compositions of the investigated species are presented in Table 4. *S. brevistigma* lacked lauric and arachidic acids. Both *A. cymosa* and *C. carandas* contained 114 gkg^{-1} lauric acid. *S. brevistigma* contained 148 gkg^{-1} myristic acid. All of the investigated species contain more than 100 gkg^{-1} palmitic acid; however the concentration was highest in *T. asthmatica* with 459 gkg^{-1} followed by *C. attenuata* with 366 gkg^{-1} and *A. cymosa* with 219 gkg^{-1}. Stearic acid content ranged from 35 to 159 gkg^{-1}. All species, except *N. odorum* (white flower variety) and *C. attenuata* contained more than 250 gkg^{-1} arachidic acid. Among the unsaturated fatty acids, *N. odorum* (red and white flowered varieties) contained 308 gkg^{-1} and 426 gkg^{-1} linoleic acid respectively, while all others contained less than 160 gkg^{-1}. Fatty acid composition of oil is one of the key factors that determine the potential use of oils as an alternate fuel source such as diesel fuel for engines [27].

Table 4 – Fatty acid composition of oil of whole plants of species from the Western Ghats, India.

Species	Fatty acid composition						
	Lauric (12:0) (gkg^{-1})	Myristic (14:0) (gkg^{-1})	Palmitic (16:0) (gkg^{-1})	Stearic (18:0) (gkg^{-1})	Oleic (18:1) (gkg^{-1})	Linoleic (18:2) (gkg^{-1})	Arachidic (20:0) (gkg^{-1})
Apocynaceae							
Allamanda cathartica	40	59	139	63	38	42	369
Aganosma cymosa	114	51	219	46	117	129	325
Carissa carandas	114	53	146	93	168	120	307
Nerium odorum (red flower variety)	86	29	139	41	30	308	253
Nerium odorum (White flower variety)	69	71	151	35	127	426	121
Asclepiadaceae							
Caralluma attenuata	45	29	366	54	155	158	192
Sarcostemma brevistigma	—	148	177	159	142	87	—
Tylophora asthmatica	153	90	459	96	127	75	287

Oils containing larger quantity of unsaturated fatty acids combine with oxygen when exposed to the air and form hard film characteristics of "drying oils" [28]. Since the white flowered varieties of *N. odorum* has more unsaturated fatty acids than the saturated, such an oil could be considered as a "drying oil" and may be a prospective source of drying oils. The oil fraction of all the investigated species, except *N. odorum* (white flower variety) contained higher saturated fatty acids which is a good property of the oil when considering the potential of these species as an alternate source for conventional oil.

Melvin Calvin reported the importance of growth rate of energy yielding plants for intensive selection and breeding programs [3]. Increasing the biomass has been the fundamental objective of bioenergy plantation from the inception of the idea to get energy from plants has provided some promising results [4]. However, Mclaughlin and Hoffman [29] are of the view that biocrude content and biomass cannot be found in the same plant. The decrease in growth rate is attributed to energy expenditure during energy production. Biocrude products of plant metabolism are controlled by various mechanisms and elements.

4. Conclusions

A. cymosa which yielded 10.3% oil with 1.2% of hydrocarbon with a gross calorific value of 35.8 MJ kg^{-1} could serve as an alternate source to conventional oil in the future if properly processed. All species belonging to Apocynaceae, except *A. cathartica* contained oil yields of 4.0% or higher. *C. attenuata* yielded 3.4% hydrocarbons with a calorific value of 38.9 MJ kg^{-1} and could serve as an efficient hydrocarbon feedstocks. Other members of Asclepiadaceae yield appreciable quantities of hydrocarbon. The oil fraction of all the species, except *N. odorum* (white flower variety) could serve as an alternative to conventional oil since they contain a higher quantity of saturated than unsaturated fatty acids. Moreover, all these plants are used as herbal medicines [30]. Bio-induction studies are warranted to improve the yield of phytochemicals as carried out by Jayabalan et al. [31]. Identification and modification of genes responsible for the desired phytochemical trait could help maximize their yield which would increase their potential for use as an alternate fuel source in the future.

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